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Two cDNAs from the plant *Arabidopsis thaliana* that partially restore recombination proficiency and DNA-damage resistance to *E.coli* mutants lacking recombination-intermediate-resolution activities

Qishen Pang, John B.Hays* and Indira Rajagopal
Department of Agricultural Chemistry and Program in Genetics, Oregon State University, Corvallis, OR 97331-6502, USA

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ABSTRACT

Escherichia coli ruvC recG **mutants lack RuvC endonuclease, which resolves crossed-strand joint molecules (Holliday junctions) formed during homologous recombination into recombinant products, and an activity (RecG) thought to partially replace RuvC. They are therefore highly deficient in homologous recombination, and sensitive to UV light and chemical DNA-damaging agents, presumably because of inability to tolerate unrepaired DNA damage by recombinational mechanisms (Lloyd, R.G. (1991) *J. Bacteriol.* 173:5414–5418). We transformed these mutants with plasmids expressing cDNAs from the plant *Arabidopsis thaliana*. Selection for bacteria with increased resistance to methylmethanesulfonate yielded two cDNAs, designated *DRT111* and *DRT112* (DNA-damage-repair/toleration). Expression of these plant cDNAs, especially *DRT111*, restored conjugal recombination proficiencies in *ruvC* and *ruvC recG* mutants to nearly wild-type levels. Both plant cDNAs significantly increased resistance of both mutants to UV light and several chemical DNA-damaging agents, but did not fully correct the mutant phenotypes. Drt111 activity, but not Drt112, also increased, to nearly wild-type levels, resistance of *recG* single mutants to UV plus mitomycin C. The predicted Drt111 and Drt112 polypeptides, 383 and 167 amino acids respectively, show no similarity with one another or with prokaryotic Holliday resolvases. Both appear chloroplast targeted; Drt112 is highly homologous to *Arabidopsis* plastocyanin. *DRT111* and *DRT112* probes hybridize only to DNA from closely related plants.**

INTRODUCTION

Projected depletion of the stratospheric ozone layer is expected to significantly increase terrestrial UV-B irradiation at DNA-damaging wavelengths (1,2). This has heightened interest in the mechanisms by which green plants, which will necessarily be exposed continually to increased UV fluxes, resist DNA-damaging agents. Studies with yeast and, especially with the bacterium *E.coli*, have demonstrated that removal of photoproducts and recombinational toleration of unrepaired DNA lesions are both important resistance mechanisms (3). Excision repair and photoreactivation of UV photoproducts have been described for several plant species, including the model green plant *Arabidopsis thaliana* (4). However, there has been no strong evidence for homologous-recombination-dependent toleration processes, such as daughter-strand- gap filling (5).

The *E.coli* RecA protein mediates homologous pairing and strand exchange during recombination, yielding a crossed-strand intermediate (Holliday junction). Mutants lacking this activity are highly sensitive to DNA damage, as well as recombination-deficient. Recently, we isolated four *Arabidopsis* cDNAs that partially complemented the UV-sensitivity phenotypes of *E.coli* mutants lacking all repair and toleration responses (Pang, Q., Hays, J.B., Rajagopal, I. and Schaefer, T.S., manuscript submitted). One of these, *DRT100* (DNA-damage-repair-toleration) proved to partially complement RecA$^-$ DNA-damage-sensitivity and recombination-deficiency (Rec$^-$) phenotypes (6). The size of the predicted Drt100 protein was similar to that of bacterial RecA proteins, but there was little global homology. Simultaneously, Jagendorf and coworkers (7) isolated an *Arabidopsis* cDNA with considerable *recA* homology by a hybridization approach, but did not test it for activity in *E.coli*. Both Drt100, the putative RecA analog, and the *Arabidopsis* RecA homolog appear to be chloroplast-targeted proteins. The existence of these genes argues strongly for the importance of DNA-damage-toleration processes in plants, at least for chloroplast genomes.

Activities that resolve crossed-strand intermediates into recombinant products have been demonstrated in *E.coli* phages T4 (8) and T7 (9), in *E.coli* itself (10), and in yeast (11–13). The *E.coli* resolvase active in extracts has been identified as the product of the *ruvC* gene (14), purified to homogeneity, and

* To whom correspondence should be addressed

characterized biochemically (15). Surprisingly, *ruvC* mutants are only slightly Rec⁻, but are highly sensitive to UV light and other DNA-damaging agents (16); double mutants, *ruvC recG*, are highly Rec⁻, and even more DNA-damage-sensitive. This suggests that *recG* encodes (or controls) a resolvase-like activity, not readily detectable in crude extracts, that substitutes fairly well for RuvC in conjugal and transductional recombination, but not in DNA-damage toleration. Further evidence for the more demanding nature of the latter process is its requirement for the RuvA and RuvB proteins as well (16).

The lack of amino-acid conservation among the phage and bacterial resolvases (17) suggested that hybridization approaches were not likely to yield the corresponding plant genes. Instead, we have selected for *Arabidopsis* cDNAs that apparently complement *E.coli* RuvC⁻ RecG⁻ phenotypes. The two cDNAs isolated in this way restore recombination nearly to wild-type levels, and increase resistance to DNA-damaging agents.

MATERIALS AND METHODS

Bacteria and bacteriophages

All strains used are derivatives of *Escherichia coli* K-12. Strain AB1157 is wild-type with respect to the markers of interest here (RuvC⁺ Rec⁺ phenotype) and is also F⁻ *thi-1 his-4* Δ(*gpt-proA*) *argE3 thr-1 leuB6 kdgK1 rfbD(?) ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31* (18). Isogenic with AB1157, except as indicated, are the RuvC⁻ strain CS85, *ruvC53 eda-51*::Tn*10*, and the RuvC⁻ RecG⁻ strain N3398, *recG258*::Tn*10* mini-*kan ruvC53 eda-51* (19), and the RecG⁻ strain N2731 (20), *recG 258*::Tn*10* mini-*kan*. Strain EG333 is HrfC *pyrA*::Tn*10 mstB cysG303* Δ(*lac-pro*)XIII (21). The vector phage λYES is *cI857*; it incorporates a cloning/expression site and yeast-*E.coli* shuttle-vector-plasmid elements between two phage P1 *lox* sites (25). Phage λCRE *cI*(*ind*⁻) *red3 xis1* overexpresses the P1 Cre protein via a bacterial *lac* promoter (6,25). Neither λYES nor λ CRE *cI*(*ind*⁻) prophages are inducible by DNA-damaging agents.

Plasmids

Plasmid pSE936, the product excised from λYES by Cre-*lox* recombination, encodes ampicillin-resistance and plasmid *ori* elements for selection and propagation in *E.coli*, as well as *URA3* and other elements for function as a yeast plasmid (25). Depending on their orientation, cDNAs inserted at the unique *Xho*I site are transcribable via the bacterial p_{lac} or yeast p_{GAL1} promoters. In plasmids pQP1110 and pQP1120, *Arabidopsis* cDNAs *DRT111* and *DRT112* are transcribed via p_{lac}. Plasmids pQP1112 and pQP1122, in which *DRT111* and *DRT112* are inverted with respect to p_{lac}, were constructed by digestion of plasmids pQP1110 and pQP1120 with *Xho*I endonuclease and re-ligation of the products, and were identified by restriction analysis of plasmids from transformed bacteria.

Media and solutions

TBY-broth, LB-broth-plates, and M9-minimal-plates have been described (26,27). TBY-Ap broth and LB-Ap plates contain 50 μg/ml ampicillin (Ap).

Selection and isolation of *DRT111* and *DRT112* cDNA

We infected about 10^{10} RuvC⁻ RecG⁻ bacteria (strain N3398), lysogenic for λCRE *cI*(*ind*⁻) *red3 xis1*, with an aliquot (5×10^{10} plaque-forming units) of an *Arabidopsis* cDNA library in the vector λYES. This library had been obtained from R. Davis, Stanford University (25) and amplified once, as described previously (6). We grew the infected cells for one hour in TBY broth, at which point there were 5×10^9 total Ap-resistant bacteria (as determined by plating a small aliquot). We selected for cells containing excised plasmids, by growth for three hours in 100 ml TBY-Ap broth, plus 2 mM isopropylthio-β-D-galactopyranoside (IPTG), yielding about 10^{11} bacteria. These were harvested by centrifugation and resuspended in 10 ml TBY. We spread the entire culture on twenty LB plates containing 0.06% methylmethane-sulfonate (MMS) and IPTG, and incubated them 40 h at 30°C. Although none of 10^{11} bacteria in a parallel N3398(pSE936) culture survived on these plates, the cDNA-library-containing culture yielded 25 survivors. These were streaked across LB plates and tested for resistance to 5, 10, 15, and 20 J/m² of UV light. Four isolates were UV-resistant, and plasmids extracted from each of these conferred resistance upon naive RuvC⁻ RecG⁻ bacteria. When digested with *Eco*RI endonuclease, one active plasmid released insert fragments of about 1.1 kb and 0.3 kb; we designated the cDNA as *DRT111*. The other three plasmids released 0.8-kb inserts; based on their apparently identical sizes, and complementation phenotypes in preliminary experiments (data not shown), we designated all three cDNAs as *DRT112*, and arbitrarily picked one for further study. (Their identity was subsequently confirmed by DNA sequence determinations.) We designated the respective plasmids pQP1110 and pQP1120.

Measurement of bacterial resistance to DNA-damaging agents

Cells were grown to late log phase in TBY-Ap broth containing IPTG, harvested by centrifugation, and resuspended to 1.5×10^8 colony-forming units (CFU) per ml, as described (6). Cell suspensions were treated with 254-nm UV light at a rate of 1 w/m² and spread on LB-Ap plates, or spread on LB-Ap plates containing mitomycin C or methylmethanesulfonate (MMS) or 4-nitroquinoline-N-oxide (NQO). All manipulations were performed under room lighting, so that all cells were phenotypically Phr⁺. Plates were incubated overnight at 30°C.

Measurement of conjugal recombinant frequencies

Procedures were essentially as described by Miller (27). Overnight cultures of the donor strain (EG333), grown in TBY broth containing tetracycline (12.5 μg/ml), and of recipient strains harboring various plasmids, grown in TBY-Ap broth with or without 2mM IPTG, were subcultured in fresh broth, grown to about 2×10^9 cells per ml, mixed at a ratio of three donors to one recipient, and incubated at 37°C. After 1 hr we stirred mixtures vigorously, harvested the cells by centrifugation, and resuspended them in one volume of 0.01M $MgSO_4$. After 30 min at room temperature, cells were spread on LB-Ap plates and incubated overnight at 37°C, to score total recipient colony-forming units, or spread on M9-glucose-minimal plates containing ampicillin (50 μg/ml), histidine (0.5 mM), arginine (0.06 mM), and proline (2 mM), to score Ap-resistant Leu⁺ Thr⁺ recombinants, or on M9-galactose-(casamino acids) plates containing ampicillin to score Ap-resistant Gal⁺ recombinants, or on M9-glucose-minimal plates containing arginine (0.06 mM), histidine (0.5 mM), proline (2 mM), ampicillin and 13 μg per ml tetracycline (Tc) to score Ap-resistant (Leu⁺ Thr⁺) recombinants.

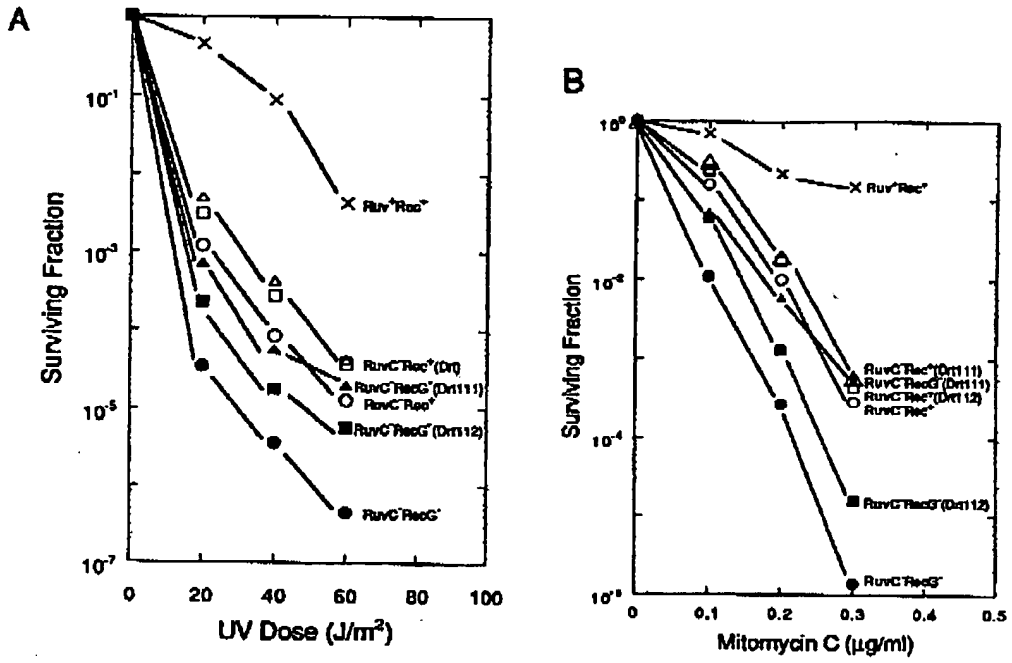

Figure 1. Resistance to DNA-damaging agents of bacteria expressing Drt111 and Drt112. Indicated bacteria were (A) irradiated with 254-nm UV light to fluences indicated, or (B) spread on plates containing indicated mitomycin C concentrations, and surviving bacteria scored, as described under 'Materials and Methods'. Surviving fraction equals CFU surviving divided by CFU on LB-Ap plates (no UV). Strains [phenotypes] employed: (×), AB1157 (pSE396) = [Ruv⁺ Rec⁺]; (○), CSR5 (pSE396) [= RuvC⁻ Rec⁺]; (△), CS85 (pQP1110) = [RuvC⁻ Rec⁺(Drt111)]; (□), CS85 (pQP1120) = [RuvC⁻ Rec⁺(Drt112)]; (●), N3398 (pSE396) = [RuvC⁻ RecG⁻]; (▲), N3398 (pQP1110) = [RuvC⁻ RecG⁺(Drt111)]; (■), N3398 (pQP1120) = [RuvC⁻ RecG⁻(Drt112)]. Data correspond to averages for two trials. Standard deviations were generally less than symbol sizes and were almost always 10% of values shown or less.

Determination and analysis of DNA sequences

The Oregon State University Central Services Laboratory determined DNA sequences on double-stranded DNA by an automated technique, using an Applied Biosystems Model 373A DNA sequencer and a Taq Dye Primer Cycle Sequencing Kit, with dideoxy chain termination, thermal cycling and primer-coupled dyes, according to instructions supplied by the manufacturer (bulletin No. 237605). M13mp*lac* Universal and Reverse primers (United States Biochemical Corp.) and internal primers were employed. For each sample, sequences were determined in both directions at least once each. In some regions we also determined DNA sequences manually, by dideoxy sequencing of duplex DNA using a Sequenase kit (United States Biochemical Corp). We compared the predicted amino-acid sequences for Drt111 and Drt112 to protein sequences in the SWISSPROT data base, using Intelligenetics Suite release 5.4 programs QUEST, PEP, SEQ, and GENALIGN. The last program, developed by Dr. H. Martinez, is a copyrighted product of Intelligenetics, Inc. We searched GENBANK release 69, and NBRFPIR and EMBL protein-sequence libraries using the Intelligenetics FASTDB search program.

Hybridization analyses

DNA was extracted from *Arabidopsis*, broccoli and cabbage (*Brassica pekinensis*) tissues as described (4). DNA from bean (*Phaseolus vulgaris*) and maize (*Zea mays*) were gifts respectively of David Mok and Carol Rivin, Oregon State University. DNA digested with *Eco*RI endonuclease was analyzed by electrophoresis, blotting, hybridization with a [^{32}P]-labeled [random-primer-method (28)] *Eco*RI fragment isolated from plasmids QP1111 and pQP1121, and autoradiography, as described (29), with minor modifications. Hybridization, at 37°C, employed solutions containing 50% formamide and 0.6 M salt. Reduced-stringency hybridization employed 32% formamide. Aqueous washes (0.03 M salt) were at 37°C; filters were autoradiographed for 48 hr.

RESULTS

Selection and isolation of cDNAs

E.coli ruvC recG bacteria lack activities that resolve recombination intermediates (Holliday junctions), and therefore are deficient in homologous recombination (Rec⁻) and DNA-damage-toleration functions (19). We established an *Arabidopsis* cDNA plasmid expression library in RuvC⁻ RecG⁻ bacteria harboring a λCre prophage, by infecting 10^{10} cells of strain N3398 with a phage λYES *Arabidopsis* cDNA library, at a multiplicity of 5 phage per cell. The endogenous Cre activity expressed by the λCre prophage (25) excised plasmids from the λYES phage via site-specific recombination at the *lox* sites in λYES; plasmids were established in about 50% of the bacteria.

We amplified cDNA-plasmid-containing bacteria and plated them in the presence of 0.06% methylmethanesulfonate, a concentration which killed all non-cDNA-containing bacteria, and

Table I. Effects of Drt111 and Drt112 on resistance of *E.coli* mutants to UV light

Relevant bacterial (plasmid) phenotype[a]	Relative survival (%) after indicated UV fluences (J/m²)[b]		
	20	40	60
Ruv⁺ Rec⁺ (none)	(100)	(100)	(100)
Ruv⁺ RecA⁻ (none)	0.3	0.05	0.05
Ruv⁺ RecA⁻ (Drt111)	0.4	0.05	0.05
Ruv⁺ RecA⁻ (Drt112)	0.4	0.06	0.05
Ruv⁺ RecB⁻C⁻F⁻ (none)	0.2	0.08	0.05
Ruv⁺ RecB⁻C⁻F⁻ (Drt111)	0.2	0.08	0.08
Ruv⁺ RecB⁻C⁻F⁻ (Drt112)	0.3	0.07	0.06
RuvC⁻ RecG⁻ (none)	0.1	0.01	0.03
RuvC⁻ RecG⁻ (Drt111)	2.7	0.16	0.67
RuvC⁻ RecG⁻ (Drt112)	1.4	0.06	0.27

[a]Respective bacterial strains (plasmids) employed, lines 1 through 10, were AB1157 (pSE936), QP3070(pSE936), QP3070(pQP1110), QP3070(pQP1120), JH312(pSE936), JH312(pQP1110), JH312 (pQP1120), N3398 (pSE936), N3398 (pQP1120), N3398 (pQP1120).
[b]Fractions of bacterial suspensions surviving indicated UV doses were measured as described under 'Materials and Methods,' and divided by surviving fractions for Ruv⁺ Rec⁺ (none) bacteria. These latter values (relative survival of 100%) were 0.32 at 20 J/m², 0.043 at 40 J/m², and 0.0027 at 60 J/m². Data correspond to averages for two plates (range typically ± 10%).

we tested the 25 survivors for resistance to 10–30 J/m² UV light. All four UV-resistant isolates harbored plasmids containing putative cDNA inserts—one of 1.4 kb, three of 0.8 kb. On the basis of the apparent identity of size and correction activity (see below) of the latter three cDNAs, we identified two unique DNA-damage-repair/toleration cDNAs, *DRT111* and *DRT112*. We tested these for their effect on various DNA-damage-sensitivity and recombination-deficiency phenotypes of *E.coli ruvC*, *ruvC recG*, and *recG* mutants.

Partial correction of DNA-damage-sensitivity phenotypes by Drt111 and Drt112

We measured the effect of Drt111 and Drt112 activity on the resistances, to ultraviolet light (Fig. 1A) and to the DNA-crosslinking agent mitomycin C (Fig. 1B), of RuvC⁻ single and RuvC⁻ RecG⁻ double mutants. Drt111 and Drt112 increased the resistance to UV light of both RuvC⁻ single mutants (Fig. 1A, open symbols) and RuvC⁻ RecG⁻ double mutants (filled symbols). Although the factors by which survival was increased were greater for the double mutants, apparent correction efficiencies relative to wild-type resistance ranged from 0.1% to 1%. Resistance of RuvC⁻ and RuvC⁻ RecG⁻ bacteria to mitomycin C (Fig. 1B; open, filled symbols) was also increased by the presence of Drt111 and Drt 112. Here resistances were increased by as much as 400-fold [RuvC⁻ RecG⁻ (Drt111) at 0.3 μg/ml], and apparent correction efficiencies were as high as 10 to 40% (at 0.1 μg/ml). The two plant cDNAs also increased resistance to methylmethanesulfonate, at concentrations of 0.015 to 0.045%, by factors of about 2-fold for RuvC⁻ and up to 20-fold for RuvC⁻ RecG⁻ mutants, corresponding to complementation efficiencies of 0.1% or less; resistance of RuvC⁻ RecG⁻ mutants to 10 μM nitroquinoline oxide was increased 7-fold by Drt111 and 3-fold by Drt112 (data not shown).

Drt111 and Drt112 might partially correct the DNA-damage-sensitive phenotypes of RuvC⁻ RecG⁻ bacteria by resolving intermediates generated by normal (RecA-dependent) *E.coli* recombinational toleration processes, or suppress the phenotypes.

Table II. Resistance of RecG⁻ mutants expressing Drt111 or Drt112 to UV light plus mitomycin C.

Relevant bacterial (plasmid) phenotype[a]	Survival (%) of bacteria treated as indicated	
	UV only	UV plus mitomycin C
Rec⁺ (none)	15 ± 2	13 ± 0.5
RecG⁻ (none)	1.7 ± 0.3	0.26 ± 0.13
RecG⁻ (Drt111)	8.5 ± 0.7	6.1 ± 0.7
RecG⁻ (Drt112)	2.7 ± 0.2	0.45 ± 0.06

[a]Respective strains (plasmids) employed, lines 1 through 4, were AB1157 (pSE936), N2731 (pSE936), N2731 (pQP1110), N2731 (pQP1120).
[b]Bacteria were grown in broth containing ampicillin and IPTG, treated with 30 J per m² UV light, as described under 'Materials and Methods,' spread on LB plates with or without 0.2 μg per ml mitomycin C (20), and incubated overnight. Data represent averages for two trials (two plates per trial), with ranges indicated.

Table III. Conjugal recombinant frequencies

Relevant bacterial (plasmid) phenotype of recipients[a]	Number of recipients (CFU/ml × 10⁻⁷)[b]	Number of Thr⁺ Leu⁺ Ap[r] recombinant (CFU/ml × 10⁻⁷)[b]	Relative recombinant frequency[c]
Rec⁺ Ruv⁺ (none)	35 ± 4	5.6 ± 0.6	(1.0)
Rec⁺ RuvC⁻ (none)	32 ± 4	1.0 ± 0.4	0.21
Rec⁺ RuvC⁻ (Drt111)	21 ± 2	2.5 ± 0.2	0.75
Rec⁺ RuvC⁻ (Drt112)	24 ± 2	1.7 ± 0.2	0.44
RecG⁻ RuvC⁻ (none)	37 ± 2	0.019 ± 0.004	0.003
RecG⁻ RuvC⁻ (Drt111)	38 ± 6	0.67 ± 0.02	0.110
RecG⁻ RuvC⁻ (Drt112)	44 ± 8	0.41 ± 0.7	0.058

[a] Respective recipient bacterial strains (plasmids) employed lines 1 to 7, were: 1), AB1157 (pSE936), CS85 (pSE936), CS85 (pQP1110), CS85 (pQP1120), N3398 (pSE936), N3398 (pQP1110), N3398 (pQP1120).
[b] Conjugal matings and measurements of total recipients and Ap-resistant Thr⁺ Leu⁺ transconjugants were performed as described under 'Materials and Methods'. Hfr donor was strain EG333. Data are averages and standard deviations for two trials.
[c] Relative recombinant frequency equals ratio of recombinant frequency to recipient frequency for indicated bacteria, divided by ratio for Rec⁺ Ruv⁻ bacteria.

by mediating new repair or recombinational toleration pathways that did not require RuvC or RecG function, for example. We tested the ability of plasmids expressing Drt111 or Drt112 to increase the resistance of other Rec⁻ mutants to UV light (Table I). Under conditions where Drt 111 and Drt112 significantly increased survival of RuvC⁻ RecG⁺ bacteria (Table I, lines 8–10), there was no effect on survival of RecA⁻ (lines 2–4) or RecB⁻C⁻F⁻ (lines 5–7) bacteria.

We also tested for correction of Rec G⁻ single mutations, using the UV-plus-mitomycin-C assay of Lloyd and Buckman (20) (Table II). RecG⁻ (Drt111) bacteria were about half as resistant as wild-type, but RecG⁻ (Drt112) bacteria were not significantly more resistant than RecG⁻.

Correction of recombination deficiencies

Although Drt111 and Drt112 both significantly increased the resistance of both RuvC⁻ and RuvC⁻ RecG⁻ mutants to a variety of DNA-damaging agents, the apparent correction efficiencies were only several percent or less, in all but a few cases. Furthermore, these data provide no direct evidence that recombination-enhancing activities are involved. Therefore, we tested the effects of Drt111 and Drt112 on a bacterial

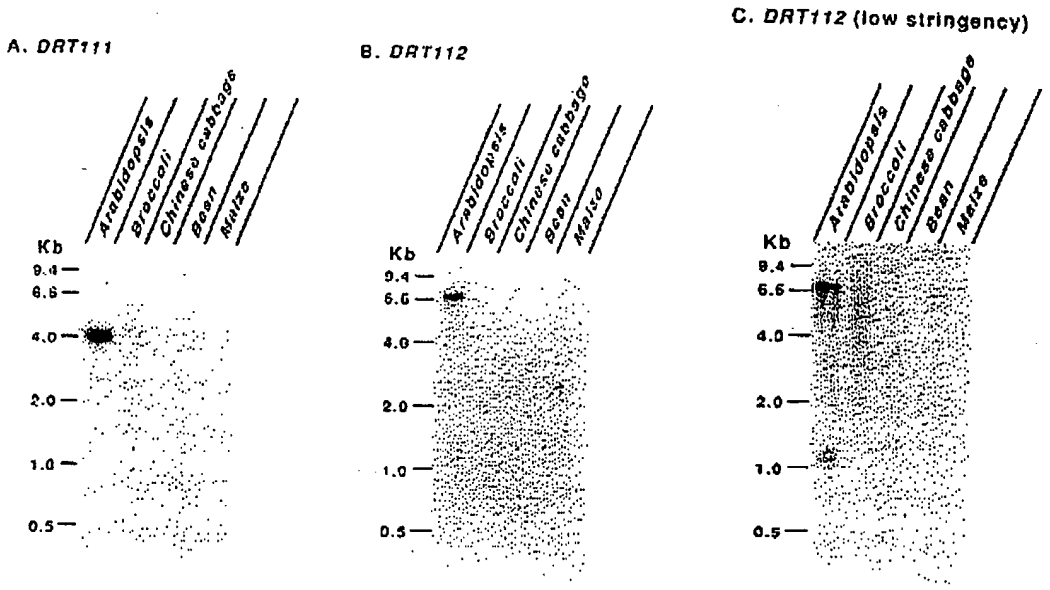

Figure 2. Hybridization of *DRT111* and *DRT112* cDNAs to plant DNA. Lanes contain DNA from *Arabidopsis* leaves and stems (5 μg), broccoli florets (10 μg), Chinese cabbage (*Brassica pekinensis*) leaves (10 μg), bean (*Phaseolus vulgaris*) seedlings (10 μg), and whole maize (*Zea mays*) plants (10 μg). Extraction of DNA, digestion with *EcoRI* endonuclease and hybridization were as described under 'Materials and Methods'. A. *DRT111* probe. B. *DRT112* probe. C. *DRT112* probe at low stringency.

recombination process. Homologous recombination is needed for formation of stable transconjugants during mating of Hfr and F⁻ bacteria. Although *recA* mutations drastically reduce conjugal recombination frequencies (30), a single *ruvC* mutation in a wild-type background reduces recombination only 5-fold [ref. (19) and Table I, line 2], perhaps because recombination intermediates are resolved in other ways. Drt111 and Drt112 corrected this RuvC⁻ phenotype, restoring recombination to nearly wild-type levels in the former case (Table III, lines 3,4). The recombinant frequency in *ruvC recG* double mutants is drastically reduced, to just 0.3% of wild-type levels (Table III, line 5). Here Drt111 increased the frequency 32-fold, to 11% of wild-type levels (about half the restoration by RecG alone), and Drt112 was slightly less effective (Table III, lines 6,7).

Neither Drt111 nor Drt112 corrected recombination deficiencies in other Rec⁻ mutants tested: The frequency of tetracycline-resistant transconjugants, in matings of the Hfr *pyrA*::Tn*10* strain EG333 with RecA⁻ and RecB⁻C⁻F⁻ recipients, were reduced respectively to 0.01% and 1% of *rec*⁺ levels, in the presence or absence of *DRT111* or *DRT112* (data not shown); in parallel experiments Drt111 and Drt112 increased recombinant frequencies in RuvC⁻ RecG⁻ bacteria from 0.17% of wild-type frequencies to 7.4% and 2.5%, respectively. Neither Drt111 nor Drt112 affected the efficiency of transfer of conjugal F′ episomes in any bacteria tested [Rec⁺, RecA⁻, RecB⁻F⁻ and RuvC⁻ RecG⁻ (data not shown)], i.e. the apparent increases in recombinant frequency are not due to increased mating efficiencies in the presence of Drt111 or Drt112.

To determine whether correction of the RuvC⁻ RecG⁻ recombination deficiency required expression of *DRT111* and *DRT112*, rather than being the result, for example, of induction of new *E.coli* activities by the presence of the plant DNA sequences themselves, we measured requirements for transcription (Table IV). Neither *DRT111* nor *DRT112*, when inverted with respect to the plasmid P_{lac} promoter, showed any correction activity (Table IV, lines 4, 6). In the absence of induction by IPTG, the activity of P_{lac}-transcribed *DRT111* and *DRT112* sequences was decreased but still significant (Table IV, lines 3, 5), presumably because multiple copies of the *lac* operator titrated out endogenous levels of even *lacI*ᵠ-expressed *lac* repressor (31).

Table IV. Effects of gene orientation and induction of expression on activity of *DRT111* and *DRT112* in recombination-deficient bacteria

Bacterial (plasmid) phenotype of recipient[a]	Frequency (%) of Ap^r Gal$^+$ transconjugants per recipient[b]	
	IPTG-induced	no IPTG
Ruv$^+$ Rec$^+$ (none)	5.0	6.2
RuvC$^-$ RecG$^-$ (none)	0.008	0.006
RuvC$^-$ RecG$^-$ (Drt111)	0.462	0.080
RuvC$^-$ RecG$^-$ (Drt111 INV)	0.008	0.008
RuvC$^-$ RecG$^-$ (Drt112)	0.180	0.060
RuvC$^-$ RecG$^-$ (Drt112 INV)	0.008	0.007

[a]Respective bacterial strains (plasmids) employed, lines 1 to 6, were AB1157 (pSE936), N3398 (pSE936), N3398 (pQP1110), N3398 (pQP1112), N3398 (pQP1120), N3398 (pQP1122).
[b]Conjugal matings, with EG333 as Hfr donor and scoring for Ap^r Gal$^+$ recombinants, were performed as described under 'Materials and Methods.' Data correspond to average for two plates; range was less than ± 10% in almost all cases.

DNA and protein sequence analyses

DNA and predicted protein sequences for *DRT111* and *DRT112* are available via GENBANK access numbers M98455 and

M98456, respectively. We used the Intelligenetics FAST DB program to search protein sequences in the GENBANK, NBRFPIR, and EMBL libraries for amino-acid-sequence similarities. The *DRT111* open reading frame predicts a 383-residue polypeptide without significant global similarity to any known protein, including the prokaryotic Holliday-junction resolvases—phage T4 gene *49* protein (32), phage T7 gene *3* protein (9), *E.coli* RuvC (17) and RecG proteins (16)—and the yeast cruciform-cutting enzyme (33). However, the Drt111 amino-acid sequence Q_{225}GqGlGKS strongly resembles the RecG sequence QGdvGsGKT (16), and thus the nucleotide binding motif (Walker box) GxxGxGKS (34) characteristic of many ATP-hydrolyzing DNA repair and recombination proteins. Surprisingly, the predicted Drt112 polypeptide, 167 amino acids, is 75% identical with *Arabidopsis* plastocyanin (35). The homology extends over most of the sequences, except for nine plastocyanin residues between Drt112 amino acids 24 and 27. Drt112 shows no significant homology with any other proteins in the data bank, or with other resolvases. The N-terminal portions of both polypeptides show features characteristic of chloroplast transit peptides (36): high frequency of serine and threonine residues, large numbers of small hydrophobic amino acids, net positive charge. We did not find a consensus chloroplast processing site (36,37) in Drt111, but the plastocyanins (35) are cleaved at a sequence that ends in Drt112 at amino acid 68. The apparent chloroplast-targeting domain of Drt111 occupies about 90 N-terminal residues.

Hybridization of *DRT111* and *DRT112* probes to plant DNA

We hybridized, at high stringency, *DRT111* and *DRT112* probes to bulk DNA from *Arabidopsis*, the closely related *Brassicaceae* broccoli and Chinese cabbage, and from bean and the monocot plant maize (Fig. 2). The *DRT111* probe hybridized strongly to DNA from *Arabidopsis* and very weakly to broccoli DNA, but not to DNA from other plants (Fig. 2A). At high stringency, the *DRT112* probe yielded only a single strong DNA signal (Fig. 2B) which presumably corresponds to *DRT112* itself. The additional two lower-molecular-weight bands that appear at lower stringency may correspond to the *Arabidopsis* plastocyanin gene (35), which encodes an *EcoRI* restriction site not in the *DRT112* sequence. Vorst *et al.* (35) detected only a single (intron-less) plastocyanin gene by hybridization analysis. Thus, despite the high degree of homology, the nucleotide differences (about 30%) apparently prevent *DRT112*-plastocyanin hybridization at high stringency.

DISCUSSION

We have isolated two *Arabidopsis* cDNAs that appear to increase recombinant progeny in conjugal crosses involving *E.coli* mutants lacking ability to resolve intermediates (Holliday structures). These cDNAs were originally selected by virtue of their ability to promote survival of *ruvC recG* mutants, on plates containing methylmethanesulfonate (MMS), but they proved to significantly increase resistance to other DNA-damaging agents as well. We have considered three explanations, other than genuine complementation—replacement of RuvC or RecG resolution activities—for these observations. Informational suppression can be ruled out: *recG258*, which is strongly corrected by *DRT111*, is an insertion mutation; the *ruvC53* allele encodes a highly temperature-sensitive but full-length protein (R. Lloyd, personal communication). Second, the requirement for P_{lac}-initiated transcription of *DRT111* and *DRT112* excludes the possibility that either DNA sequence in and of itself provokes a phenotype-suppressing response in *E.coli*. Third, the inability of Drt111 or Drt112 to promote conjugal recombination or UV-resistance in *E.coli recA* or *recB recC recF* mutants argues strongly against the notion that either activity mediates a novel recombination pathway that does not depend on resolution via RuvC or RecG activity.

Correction efficiencies, for *DRT111* and *DRT112* relative to wild-type cells, were high for conjugal recombination (9–70% and 4–25%, respectively), for resistance to lower levels of mitomycin C (10–40% and 3–10% respectively), and for complementation by Drt111 of RecG$^-$ DNA-damage sensitivity (50%). Efficiencies were less for higher degrees of damage. These trends may reflect saturation, by high amounts of DNA damage, of activities limited by lack of bacterial translation signals, poor codon usage, RNA instability or protein instability, or insolubility in *E.coli*, or the presence of activity-inhibiting chloroplast processing signals on the Drt111 and Drt112 polypeptides. In fact, in experiments in which proteins were radiolabeled in *E.coli* 'mini-cells', neither Drt111-encoding nor Drt112-encoding plasmids yielded detectable bands of the appropriate molecular weight (Q. Pang, unpublished results). The correction patterns thus suggest that activities able to efficiently resolve a few conjugal-recombination intermediates, despite low intrinsic biochemical proficiency and/or low levels of expression, may not be able to deal with large numbers of intermediates arising during DNA-damage-provoked sister-chromatid exchange.

Drt111 and Drt112 differ in the sequences and length of their polypeptides, their apparent efficiencies for complementation of RuvC$^-$ and RuvC$^-$ RecG$^-$ phenotypes, and in the ability of Drt111, but not Drt112, to efficiently complement a RecG$^-$ phenotype. The molecular weight of Drt111, 42 kDa, falls between those of RecG, 76 kD (16) and RuvC and the phage T7 and T4 resolvases, 17–19 kDa (8–10). Drt112 is highly similar to *Arabidopsis* plastocyanin (35), a nuclear-encoded chloroplast protein that participates in electron transfer between photosystem I and the cytochrome *b/f* complex. If Drt112 is processed at the same site at which plastocyanin is thought to be cleaved in the chloroplast (35), the putative nature Drt112 protein (amino acids 69–167), would have a molecular weight of only 11 kDa, significantly less than those of the RuvC/phage resolvases. The Drt112-plastocyanin similarity is reminiscent of the similarity in between respiratory-chain NADH dehydrogenase and a protein that binds to the chloroplast DNA replication origin (38).

E.coli appears to process recombination intermediates, such as Holliday structures, in two steps, by at least two pathways. The bacterial RuvA and RuvB proteins together recognize Holliday junctions and catalyze ATP-dependent branch migration (39,40,41); RecG alone efficiently accomplishes both these tasks (42). Both RuvAB and RecG activities resolve Holliday junctions in short linear model substrates, simply via branch migration out to the ends. However, resolution of such intermediates in chromosomes would require that the migrating junctions encounter preexisting DNA strand nicks, or be cleaved by resolving endonucleases. The RuvC protein is one such resolvase (10,14). Since RuvC$^-$ (and RuvA$^-$ or RuvB$^-$ mutants) are only slightly recombination-deficient (19), yet RecG does not appear to cleave Holliday junctions (42), there may another, as yet unidentified, *E.coli* resolvase that cleaves junctions recognized

by RecG. Preference of the RuvC resolvase for RuvAB-bound Holliday junctions has not been demonstrated (39,40,41). However, the indistinguishability of RuvA$^-$, RuvB$^-$, and RuvC$^-$ phenotypes (19) suggests that these proteins, which are encoded by three nearly contiguous genes (17), may act cooperatively *in vivo*. Ruv$^-$ mutants are much more sensitive to DNA damage than RecG$^-$ mutants, although both classes of mutants are only slightly recombination-deficient (19). This suggests that the Ruv and RecG pathways are nearly, but not perfectly, interchangeable for conjugal recombination, but that the Ruv proteins are designed to play the predominant role in recombinational toleration of DNA damage.

How might the *Arabidopsis* Drt activities identified here relate to these *E.coli* functions? Drt111 is slightly more than half the size of RecG. However, both proteins exhibit a nucleotide binding site, recombination frequencies in RuvC$^-$RecG$^+$ and RuvC$^-$RecG$^-$ (Drt111) bacteria are similar (Table III), and Drt111 efficiently corrects RecG$^-$ phenotypes, despite its apparently low level of expression. Although Drt111 may thus serve as a RecG analog, Drt112 does not correct RecG$^-$ phenotypes. The pattern of correction, by apparently limited amounts of Drt112, of RuvC$^-$ and RuvC$^-$RecG$^-$ phenotypes is not inconsistent with partial replacement of the RuvC endonuclease, but such an identification requires further study, *in vitro* as well as *in vivo*. The lack of extensive similarity among all proteins thus far implicated in resolution of recombination intermediates—Drt111, Drt112, the *E.coli* Ruv and RecG proteins, and the phage T4 and T7 resolvases—suggests that various organisms have recruited a wide variety of proteins to mediate this process. The validity of these speculations remains to be tested, by biochemical studies with purified Drt111 and Drt112 proteins.

The two *Arabidopsis* RecA homologs/analogs described previously (6,7), and the apparent plant resolution proteins described here, incorporate putative chloroplast transit peptides. This suggests that recombinational toleration is an important feature of resistance of chloroplasts to DNA damage.

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